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were estimated from both intravenous dosing studies and an inhalation study with TCE. For the pup, K (0.014 ± hr^{-1}) and V_d (0.511 liter/kg) were estimated from a single 4-hr inhalation exposure with TCE. The dose-rate-dependent stoichiometric yield of TCA from oxidative metabolism of TCE in the lactating rat is 0.17 for a low-concentration inhalation exposure (27 ppm TCE) and 0.27 for an exposure above metabolic saturation (about 600 ppm TCE). For the pup, the stoichiometric yield of TCA is 0.12. With changing physiological values during lactation for compartmental volumes, blood flows, and milk yields obtained from the published literature and kinetic parameters and PCs determined by experimentation, a PB-PK model was constructed to predict maternal and pup concentrations of TCE and TCA. To test the fidelity of the PB-PK lactation model, a multiday inhalation exposure study was conducted from Days 3 to 14 of lactation and a drinking water study, from Days 3 to 21 of lactation. The inhalation exposure was 4 hr/day, 5 days/week, at 610 ppm. The TCE concentration in the drinking water was 333 ug/ml. Prediction compared favorably with limited data obtained at restricted time points during the period of lactation.

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TOXICOLOGY AND APPLIED PHARMACOLOGY **102,** 497–513 (1990)

Physiologically Based Pharmacokinetic Modeling of the Lactating Rat and Nursing Pup: A Multiroute Exposure Model for Trichloroethylene and Its Metabolite, Trichloroacetic Acid

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Physiologically Based Pharmacokinetic Modeling of the Lactating Rat and Nursing Pup: A Multiroute Exposure Model for Trichloroethylene and Its Metabolite, Trichloroacetic Acid. FISHER, J. W., WHITTAKER, T. A., TAYLOR, D. H., CLEWELL, H. J., III, AND ANDERSEN, M. E. (1990). *Toxicol. Appl. Pharmacol.* **102,** 497–513. A physiologically based pharmacokinetic (PB-PK) model was developed to describe trichloroethylene (TCE) kinetics in the lactating rat and nursing pup. The lactating dam was exposed to TCE either by inhalation or by ingestion in drinking water. The nursing pup's exposure to TCE was by ingestion of maternal milk containing TCE. The kinetics of trichloroacetic acid (TCA), a metabolite of TCE, were described in the lactating dam and developing pup by a hybrid one-compartment model. The lactating dam's exposure to TCA was from metabolism of TCE to TCA. The pup's exposure to TCA was from metabolism of TCE ingested in suckled milk and from direct ingestion of TCA in maternal milk. For the PB-PK model, partition coefficients (PCs) were determined by vial equilibration, and metabolic constants for TCE oxidation, by gas uptake methods. The blood/air and the fat/blood PCs for the dam were 13.1 and 34.2, and for the pup, 10.6 and 42.3, respectively. The milk/blood PC for the dam was 7.1. In lactating rats and rat pups (19–21 days old) the maximum velocities of oxidative metabolism were 9.26 ± 0.073 and 12.94 ± 0.107 mg/kg/hr. The plasma elimination rate constant ($K = 0.063 \pm 0.004\ hr^{-1}$) and apparent volume of distribution ($V_d = 0.568$ liter/kg) for TCA in the lactating dam were estimated from both intravenous dosing studies and an inhalation study with TCE. For the pup, K ($0.014 \pm hr^{-1}$) and V_d (0.511 liter/kg) were estimated from a single 4-hr inhalation exposure with TCE. The dose-rate-dependent stoichiometric yield of TCA from oxidative metabolism of TCE in the lactating rat is 0.17 for a low-concentration inhalation exposure (27 ppm TCE) and 0.27 for an exposure above metabolic saturation (about 600 ppm TCE). For the pup, the stoichiometric yield of TCA is 0.12. With changing physiological values during lactation for compartmental volumes, blood flows, and milk yields obtained from the published literature and kinetic parameters and PCs determined by experimentation, a PB-PK model was constructed to predict maternal and pup concentrations of TCE and TCA. To test the fidelity of the PB-PK lactation model, a multiday inhalation exposure study was conducted from Days 3 to 14 of lactation and a drinking water study, from Days 3 to 21 of lactation. The inhalation exposure was 4 hr/day, 5 days/week, at 610 ppm. The TCE concentration in the drinking water was 333 μg/ml. Prediction compared favorably with limited data obtained at restricted time points during the period of lactation.

Trichloroethylene (TCE), a common environmental contaminant, has been listed by the U.S. EPA (1987) as a possible human carcinogen. In addition to cancer, other important biological effects resulting from TCE exposure have been noted in laboratory studies with animals and in occupational or solvent abuse exposures with humans (U.S. EPA, 1985). Recently, Taylor *et al.* (1985) and Noland-Gerbec *et al.* (1986) reported behavioral

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and biochemical effects of TCE in neonatal rats born to dams exposed to TCE via drinking water during pregnancy and lactation. The exposure of the neonates to TCE or TCE metabolites occurred both during pregnancy *in utero* and in the postpartum period by ingestion of milk containing these chemicals. These studies did not establish the fetal or neonatal dosimetry for TCE or its metabolites. In an attempt to predict fetal and neonatal dosimetry, two generic physiologically based pharmacokinetic (PB-PK) models were developed and applied to study TCE and its persistent oxidative metabolite trichloroacetic acid (TCA). One model was developed for the pregnant rat and developing fetus (Fisher *et al.*, 1989) and another for the lactating dam and nursing pup.

Quantitative methodology for estimating infant exposure to chemicals as a result of maternal exposure to chemicals is not well developed. Recently Shelley *et al.* (1989) described a physiologically based lactation model for estimating infant exposure to solvents based on the ability of the chemical to partition into various tissue groups, including the milk tissue. Wilson *et al.* (1980) described a classical three-compartment model for estimating infant exposure to drugs based on first-order clearance of the drug from the body of the lactating mother. Rodents have been used as experimental animals for studying lactational transfer of industrial chemicals such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls (Dent *et al.*, 1978; McCormack *et al.*, 1979; Spindler-Vomachka and Vodicnik, 1984). No compartmental or PB-PK models were found in the literature for the lactating rodent. Our PB-PK lactation model was developed to describe the uptake, disposition, and elimination of TCE, a well-metabolized, volatile industrial chemical, and TCA, one of its nonvolatile metabolites, in both the lactating rat and the rat pup (Fig. 1).

METHODS

Animals. Adult female cesarean-derived Fischer-344 timed-pregnant rats previously exposed to TCE during pregnancy (Fisher *et al.*, 1989) were used for repeated-exposure studies during lactation. Female cesarean-derived Fischer-344 rats, obtained from Charles River Breeding Laboratory, Kingston, New York, were used in single-exposure studies for kinetic constant determinations. All rats were kept in separate cages and allowed access to commercial rat chow (Purina Rat Chow) and water *ad libitum*.

TCE and TCA analyses. TCE (99.9%) and TCA (98%) were obtained from Aldrich Chemical Company. Methodology for preparation and analyses of TCE in blood and TCA in plasma is reported in Fisher *et al.* (1989). Milk samples obtained in this study were analyzed for TCE and TCA using the same assay procedures, except the milk samples were not centrifuged prior to analysis for TCA. Lactating rats were anesthetized with a 0.6 mg/kg ip injection of ketamine (42 mg/kg) and xylazine (3.6 mg/kg) and given oxytocin (0.25 ml/kg; 1.0 ml equals 10 USP units). Within 5 to 8 min, the rat was anesthetized and milk release occurred. The rat's teats were massaged to express milk into 0.1-ml capillary tubes.

TCA kinetic constants by iv dosing. Four jugular-cannulated lactating rats (Day 16 or 17 of lactation) were injected in the femoral vein with 4.4 mg TCA/kg in saline, and blood (0.1 ml) was collected from the indwelling cannulas at 1, 3, 12, and 23 hr postexposure. The plasma elimination rate constant for TCA in the lactating rat was calculated from log-linear regression of the TCA plasma concentration over time. Apparent volume of distribution for TCA was estimated by dividing the dose of TCA by the calculated initial concentration of TCA in the plasma.

Single inhalation exposure. A 4-hr inhalation exposure to 600.4 ppm TCE (time-weighted average, TWA) was conducted with six jugular-cannulated adult lactating rats (Day 12 of lactation). Blood (0.3 ml) was collected from the cannulated rats for analyses of TCE and TCA at 0.5 and 3.5 hr during exposure and at 0.25, 0.50, 1.0, and 2.0 hr postexposure. Additional blood samples (0.15 ml) were collected at 5, 21, 29, and 45 hr postexposure for TCA analysis. Another 4-hr inhalation exposure to 27 ppm TCE (TWA) was conducted with five noncannulated lactating rats (Day 15 of lactation). The animals were killed immediately after exposure and blood was collected for *TCE and TCA* analyses.

Two 4-hr 600.0-ppm TCE (TWA) inhalation exposures were conducted using 21 rat pups for one exposure and 30 rat pups (20 days of age in both studies) for the other exposure. For the first exposure, blood was collected by heart puncture at 0.5 hr during exposure and 0.17, 0.25, 0.50, 1.0, and 2 hr postexposure for TCE analysis. Three rat pups were killed at each of the first three time periods and four rat pups at the other time periods. For the second exposure, rat pup blood was collected by heart puncture at 0.25 hr during exposure and 0.17, 1, 2, 5, 21, 29, and 45 hr postexposure for TCA analysis. Three rat pups were killed at each time period except at 0.17 and 29 hr, at which four rat pups were killed. The

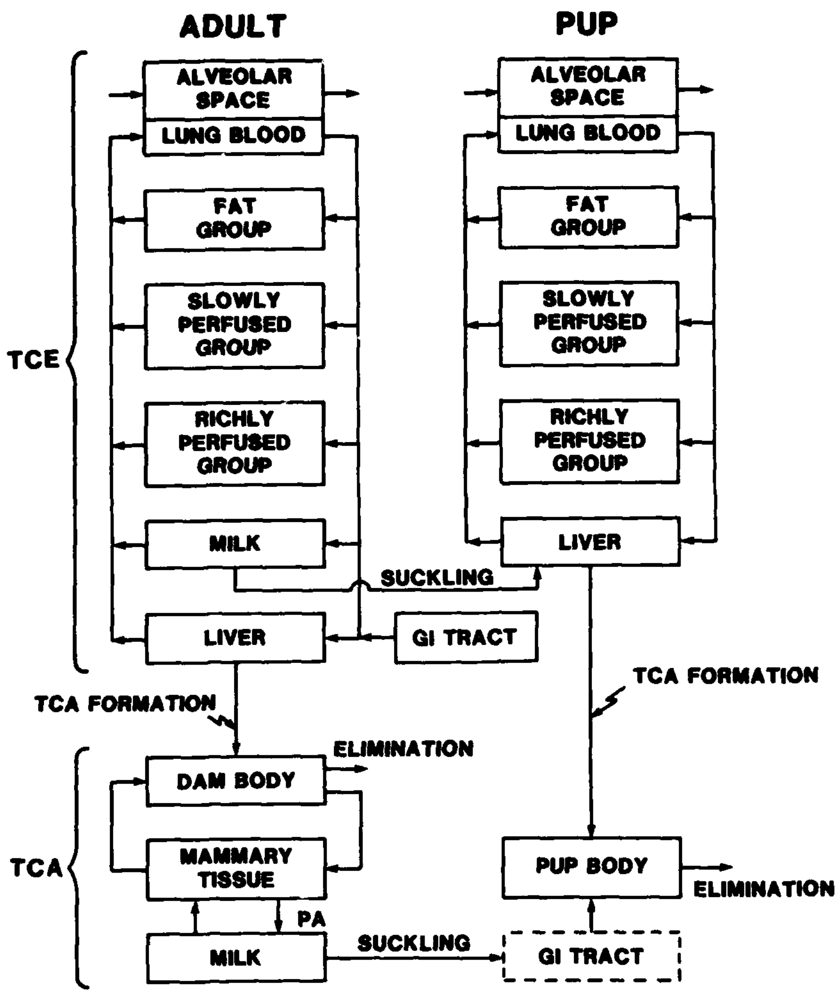

FIG. 1. A physiologically based pharmacokinetic lactation model was used to describe the disposition of TCE in the lactating rat and nursing neonate. A compartmental model was used to describe the disposition of TCA, an oxidative metabolite, in the lactating rat and nursing neonate. TCE enters the dam body by either inhalation or oral ingestion of drinking water. Rat pups are exposed to TCE and TCA from ingestion of milk containing these chemicals. TCA is formed from metabolism in the liver in both the dam and pup.

inhalation chamber and the atmospheric analysis of TCE vapor in the chamber have been described (Fisher *et al.*, 1989).

Repeated inhalation exposure. Eleven lactating rats previously exposed to 618 ppm of TCE during pregnancy (Fisher *et al.*, 1989) were exposed to 610 ppm TCE (TWA) for 4 hr/day, 5 days/week for 2 weeks (Days 3 to 14 of lactation). Three dams were milked and blood was collected for TCA analysis 20 hr after exposure on Day 11 of lactation. Blood was collected immediately after exposure from three adult rats on Day 12 of lactation and blood and milk were collected immediately after exposure from five rats on Day 14 of lactation for TCE and TCA analyses. Pups (male and female) belonging to each dam were killed at the same time as the dam and their blood was pooled for TCA analysis.

Repeated drinking water exposure. Fourteen lactating rats, previously maintained on drinking water containing 350 μg TCE/ml water during pregnancy (Fisher *et al.*, 1989), were provided drinking water containing 333.0 (SE 12.6) μg TCE/ml water, 5 days/week, for 3 weeks. Five control lactating rats received distilled water. Water consumption of the treated animals increased during lactation from 24 ml/day/rat for the first week to 37 ml/day/rat by the second week and 45 ml/day/rat on the third week of lactation. Control animals showed a similar trend. Decline in the water concentration of TCE in the drinking water bottles was monitored and the average daily loss was described by a first-order process with a rate constant of 0.039 hr^{-1}. Fresh TCE–water solutions were prepared daily. On Days 13 ($n = 5$), 14 ($n = 5$), and 21 ($n = 4$) of lactation between 10:00 and 11:00 AM, rats were milked and blood was collected for TCA analysis. TCE was assayed in the milk and blood of the dams on Day 13 of lactation. Pups (male and female) belonging to each dam were killed at the same time as the dam and blood was collected for TCA analysis.

Gas uptake. The gas uptake method (Gargas *et al.*, 1986) was used to assess total *in vivo* metabolism of TCE in the lactating rat and in the pup. Experimental exposures for kinetic constant determinations were conducted with four lactating adult rats per exposure (Days 12 to 14 of lactation) and 24 rat pups per exposure (19 to 21 days old). Lactating rats were exposed to initial TCE concentrations of 2200, 1100, and 110 ppm and rat pups to initial TCE concentrations of 2100, 1250, and 100 ppm. Experimental conditions for conducting the gas uptake studies are reported in Fisher *et al.* (1989). V_{max}, the maximum rate of metabolism (mg/hr) for a 1.0-kg animal (allometrically scaled), was estimated by computerized nonlinear least-squares techniques (Simusolv, Dow Chemical Co.). The lactation model for Day 12 of lactation was used to obtain the statistical best-fit estimates of V_{max}, while the four-compartment model structure (Ramsey and Andersen, 1984) was used for fitting V_{max} with the rat pups. Physiologic parameters for lactation are reported under Methods and reported in Table 1; TCE kinetic constants are reported in Table 2. Appendices I and II contain lactation model nomenclature and mathematical equations.

Partition coefficients. TCE tissue/air partition coefficients were determined for blood and milk using Day 14 lactating rats and for blood using rat pups 21 days of age. The vial-equilibration method (Sato and Nakajima, 1979; Gargas *et al.*, 1989) was used to determine partition coefficients. Tissue/air partition coefficients for other tissue groups were collected using homogenized tissue from adult naive female Fischer 344 rats (Fisher *et al.*, 1989). The tissue/air partition coefficient was divided by the blood/air partition coefficient to obtain the tissue/blood partition coefficient. Experimental conditions for conducting the vial-equilibration studies are reported in Fisher *et al.* (1989).

TCA is a nonvolatile chemical whose partition coefficients cannot be determined by vial equilibration methods. TCA tissue/saline partition coefficients were determined for blood and mammary tissue using a centrifugation technique (Jepson, 1986). The mammary tissue/saline partition coefficient was divided by the blood/saline partition coefficient to estimate the mammary tissue/blood partition coefficient.

Physiological parameters. Certain time-dependent physiological changes that occur during lactation (Table 1) were incorporated into the description of the lactating rat and nursing pup. These physiological changes were estimated by linear interpolation in the lactation model using the table function of the simulation software, Advanced Continuous Simulation Language (ACSL) (Mitchell and Gauthier Assoc., Inc., 1981). All tissues were assumed to have unit density.

Maternal weight gain was measured and represented in the lactation model as 12 and 16% increases in initial body weight (Day 3 of lactation) on Days 17 and 23 of lactation. Individual pup weight gain was measured from Day 3 to Day 23 of lactation, and described as the exponential function

$$\mathrm{BWP\ (kg)} = 0.0045 * \mathrm{EXP}[0.0036(t)], \quad (1)$$

where $0 \leqslant t \geqslant 504$ hr (21 days). Mammary tissue growth was based on the experimental findings of Knight *et al.* (1984). Mammary tissue weight was 4.4, 5.6, 6.3, and 9.6% of body weight on Days 3, 10, 17, and 23 of lactation, respectively. Blood flow to the mammary tissue was 9.0, 10.0, 11.0, 14.0, and 15.0% of cardiac output on Days 3, 8, 13, 18, and 23 of lactation, respectively (Hanwell and Linzell. 1973). Depletion of fat tissue which accumulated during pregnancy was described as a linear process changing from 12.0% of body weight of Day 3 of lactation to 6% on Day 23 of lactation (Naismith *et al.*, 1982). The pup litter suckling rate was assumed to equal the milk production rate. Milk production, based on experimental findings of Knight *et al.* (1984), was 0.0009, 0.0016, 0.0018, and 0.0016 liters/hr on Days 3, 10, 17, and 23 of lactation, respectively. The residual volume of milk was assumed to equal 0.002 liter.

Cardiac output and alveolar ventilation in the lactating rat were 14.0, 18.6, 19.0, and 21.0 liters/hr (allometrically scaled for 1-kg animal) on Days 3, 8, 13, and 23 of lactation, respectively (Hanwell and Linzell, 1973). For the rat pup, cardiac output and alveolar ventilation were set to 18.0 and 22 liters/hr (allometrically scaled for 1-kg animal), respectively, based on the 4-hr rat pup inhalation exposure to TCE (see Results). Development of the mixed function oxidase system in the maturing pup was based on the developmental findings of MacLeod *et al.* (1972) and our metabolic measurements (gas uptake) taken with rat pups 19 to 21 days old. The pup's ability to metabolize TCE on a per weight basis was set to 15, 24, 94, and 129% that of the lactating dam on Days 3, 9, 16, and 23 of lactation.

Physiologic constants for blood flow to the liver, fat, and slowly perfused tissue groups were given values of 25, 9, and 15% of cardiac output, respectively, for both dam and pup. Blood flow to the richly perfused tissue ranged from 41 to 36% of cardiac output for the dam and 51% for the pup. Volumes of the liver and of the slowly perfused and richly perfused tissue groups were 4, 62.6–63.4, and 8% of body weight, respectively, in dams and 4, 76, and 5%, respectively, in pups. The pup fat compartment was 6% of body weight. For the drinking water exposure the lactating rats were assumed to drink the TCE–water mixture over a 6-hr period (2400–0600 hr). Based on total daily consumption of the TCE–water mixture the lactating rats drank an average of 4.0, 6.1, and

TABLE 1

PHYSIOLOGICAL CONSTANTS USED IN THE PB-PK MODEL FOR THE LACTATING RAT AND THE NURSING PUP

	Lactating dam	Pup
	Body weight (kg)	
Single inhalation	0.192	0.025
Repeated inhalation	0.189–0.219[a]	0.0045–0.0276[b]
Repeated drinking water	0.173–0.201[a]	0.0045–0.0276[b]
	Percentage of body weight	
Liver	4.0	4.0
Richly perfused	8.0	5.0
Slowly perfused	62.6–63.4	76.0
Fat	12.0–6.0	6.0
Mammary tissue	4.4–9.6	—
Milk	0.002 kg	—
	Flows (liters/hr)	
Alveolar ventilation	$14.0\text{–}21.0 * BW^{0.74}$	$22.0 * BW^{0.74}$
Cardiac output	$14.0\text{–}21.0 * BW^{0.74}$	$18.0 * BW^{0.74}$
	Percentage of cardiac output	
Liver	25.0	25.0
Richly perfused	41.0–36.0	51.0
Slowly perfused	15.0	15.0
Fat	9.0	9.0
Mammary tissue	9.0–15.0	—

[a] Measured initial body weight on Day 3 of lactation and predicted body weight on Day 23 of lactation.
[b] Predicted individual pup weight on Days 3 and 23 of lactation. Litter size equals 7.

7.5 ml/hr for a 6-hr period for the first, second, and third weeks of lactation, respectively.

RESULTS

Partition Coefficients

The blood/air partition coefficient was slightly higher for the lactating dam (13.1 ± 0.35) than for the male and female pups 19 to 21 days of age (10.6 ± 0.79). As expected for a moderately lipophilic chemical (Table 2), TCE readily partitioned into fat and milk, which is about 15% fat (Wilson *et al.*, 1980).

Rates of Metabolism

A best-fit estimate for V_{max} was obtained by varying V_{max} with a constant K_m. At the lowest initial concentrations of 100 and 110 ppm for dam and pup, respectively, chamber loss of TCE was very rapid. Exposures at these concentrations were not included as part of the analysis. Rapid loss of TCE at low concentrations in gas uptake chambers may be due to complete clearance of inhaled TCE (Gargas *et al.*, 1986). Values of K_m below about 0.5 mg/liter had little effect on the simulations; thus, K_m was set to 0.25 mg/liter to be consistent with other gas uptake studies with TCE (Andersen *et al.*, 1987; Fisher *et al.*, 1989). As in the pregnant rat (Fisher *et al.*, 1989) the small value for K_m indicates that TCE metabolism is flow-limited for these rats at low TCE concentrations (Andersen, 1981). The best-fit estimates for V_{max} were 9.26 ± 0.073 mg/kg/hr for the lactating dam (Fig. 2A) and 12.94 ± 0.107 mg/kg/hr for the rat pup (Fig. 2B).

TABLE 2

KINETIC CONSTANTS FOR MODELING TRICHLOROETHYLENE AND TRICHLOROACETIC ACID IN THE LACTATING RAT AND NURSING PUP

	Lactating dam	Pup
Partition coefficients		
TCE		
Blood/air	13.10	10.60
Liver/blood	1.67	2.06
Rapidly perfused/blood	1.67	2.06
Slowly perfused/blood	0.53	0.65
Fat/blood	34.20	42.28
Milk/blood	7.10	—
TCA		
Mammary tissue/blood	1.10	—
Metabolic constants		
TCE		
V_{max} (mg/hr)	2.81[a]	0.84[b]
K_m (mg/liter)	0.25	0.25
P_O (unitless)	0.17 or 0.27	0.12
TCA		
V_d (liters)	0.114[c]	0.013[d]
K (hr^{-1})	0.102[e]	0.042[f]
P_A (liters/hr)	0.0005	—

[a] Body weight fixed at 0.200 kg, V_{max} (lactating rat) = $9.26*BW^{0.74}$.

[b] Body weight fixed at 0.025 kg, V_{max} (pup) = $12.94*BW^{0.74}$.

[c] Body weight fixed at 0.200 kg, V_d (lactating rat) = $0.568*BW$.

[d] Body weight fixed at 0.025 kg, V_d (pup) = $0.511*BW$.

[e] Body weight fixed at 0.200 kg, K (lactating rat) = $0.063/BW^{0.3}$.

[f] Body weight fixed at 0.025 kg, K (pup) = $0.014/BW^{0.3}$.

Pharmacokinetics of Intravenously Administered TCA

Elimination of TCA from the plasma of the lactating rat after intraveneous administration of 4.4 mg TCA/kg in saline was adequately described by a one-compartment model (data not shown). The calculated elimination rate constant (and 95% confidence interval) for TCA in plasma was 0.086 (0.070, 0.100) hr^{-1}. The apparent volume of distribution in the lactating rat was estimated to be 0.541 (0.449, 0.699) liters/kg.

Single 4-hr TCE Inhalation Exposures

The PB-PK lactation model (Fig. 1) was used to predict the TCE blood time courses in lactating rats on Days 12 and 15 of lactation and in rat pups 20 days of age following single 4-hr inhalation exposures to TCE. PB-PK modeling of the single inhalation exposures for the dam (without pups) was accomplished by setting the pup litter suckling rate during the exposure period to 0.0. A four-compartment PB-PK model (Ramsey and Andersen, 1984) was used for the description of TCE kinetics in the pup. Day 12 dams (without pups) were exposed for 4 hr to 600.4 ppm TCE and Day 15 dams (without pups) to 27 ppm TCE for 4 hr. The predicted maternal TCE blood concentration time course for the 600.4-ppm exposure was in close agreement with experimental observations (Fig. 3A). For the 27-ppm exposure, the predicted maternal blood concentration of TCE immediately after exposure was 0.20 μg TCE/ml blood and the observed concentration was 0.13 μg TCE/ml blood. Rat pups (without dams) were exposed to 600.0 ppm TCE for 4 hr. The four-compartment PB-PK model for the pup initially underestimated the rate of uptake of TCE into the pup's systemic circulation and overestimated the rate of clearance from systemic circulation. The cardiac output and alveolar ventilation rates for the pup were adjusted from 14 liters/hr to 18 and 22 liters/hr, respectively, to fit simulation with observation (Fig. 4A). The model then adequately predicted the uptake of TCE into systemic circulation for the pup, while clearance of TCE from systemic circulation was still somewhat slower than predicted. These values for alveolar ventilation and cardiac output were used for estimating the metabolic constant, V_{max}, for the pup (Fig. 2B).

In addition to validation and refinement of the TCE kinetics, these 4-hr inhalation experiments provided estimates of the yield of TCA from TCE metabolism and of the kinetic characteristics of TCA formed during these inhalation exposures in both dam (Fig. 3B) and pup (Fig. 4B). TCE oxidation results

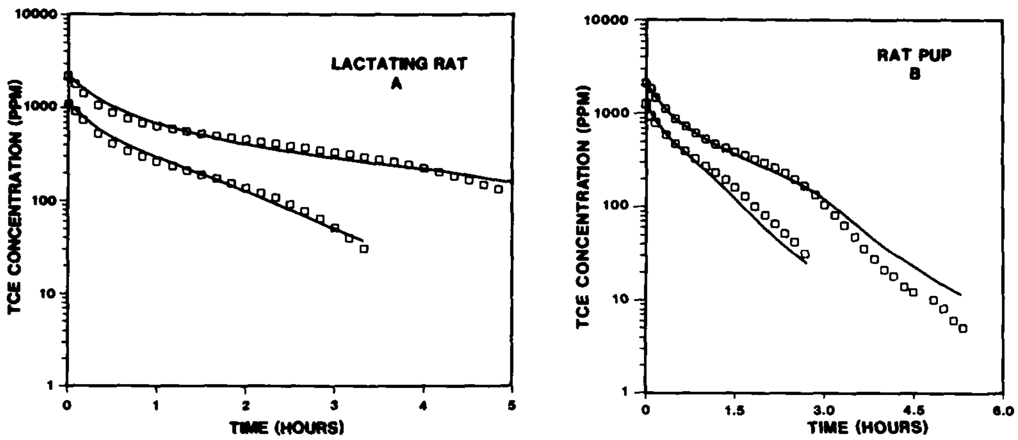

FIG. 2. Uptake of TCE from a closed recirculating atmosphere. The solid continuous curves were generated by the computer model and the squares represent experimentally determined vapor concentrations of TCE. (A) Female lactating rat (Days 12 to 14 of lactation). The initial vapor concentrations of TCE in the chamber were 2200 and 1100 ppm. Four rats were used for each exposure. (B) Male and female rat pups (19 to 21 days old) The initial vapor concentrations of TCE in the chamber were 2100 and 1250 ppm. Twenty-four rat pups were used for each exposure.

in the formation of an intermediate, trichloroacetaldehyde, which is then either oxidized to TCA or reduced to trichloroethanol. In the lactating dam the proportion of TCE oxidized to TCA was different for the two inhalation exposures. The 27-ppm exposure produced a plasma concentration of TCA near 2.8 mg/liter immediately after exposure, and the 600.4-ppm exposure, near 37 mg TCA/liter plasma. Modeling attempts in which the apparent volume of distribution and plasma elimination rate constant were held constant and P_0, the proportionality constant, was varied provided estimates of P_0. The estimated P_0 value for the 27-ppm exposure was 0.17, and that for the 600.4 ppm exposure, 0.27,

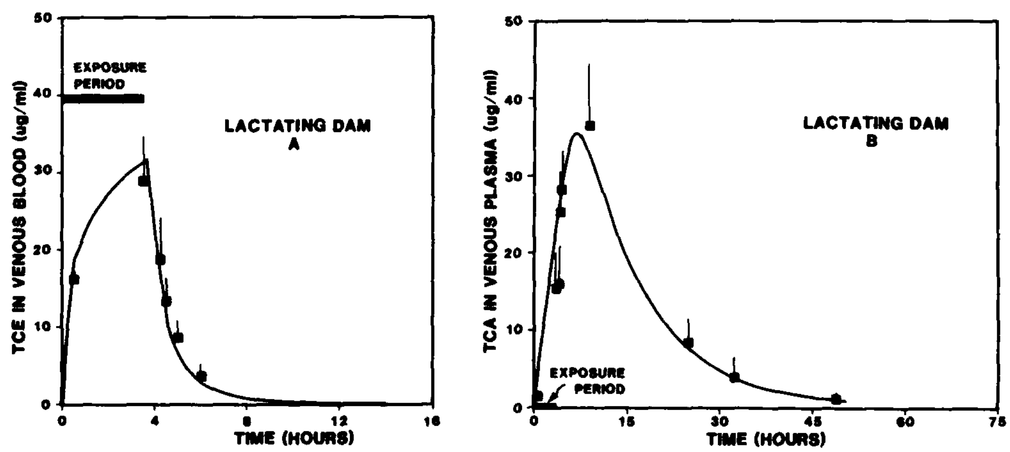

FIG. 3. Comparison of predicted (solid continuous lines) and experimental (squares) concentrations of TCE in venous blood (A) and TCA in venous plasma (B) of Day 12 lactating rats. The lactating rats were exposed to 600.4 ppm TCE vapor for 4 hr. Data points are means ± standard deviations (n = 6).

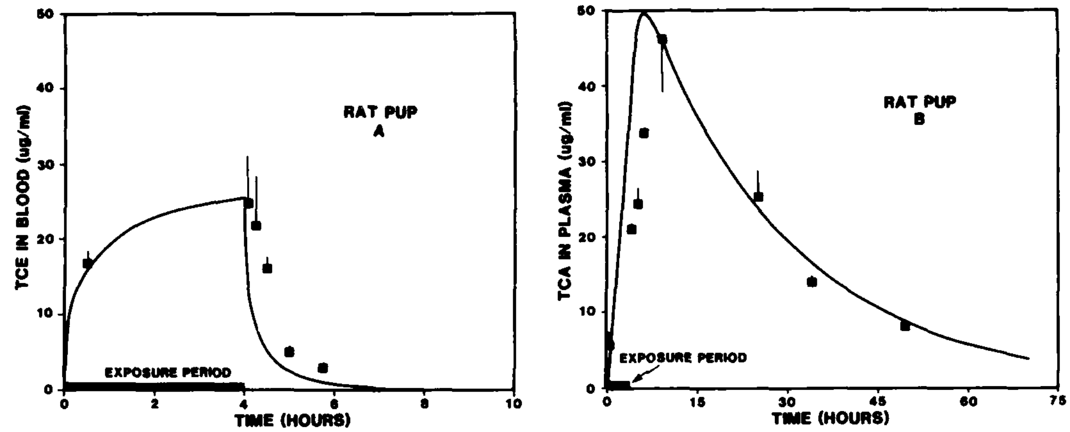

FIG. 4. Comparison of predicted (solid continuous lines) and experimental (squares) concentrations of TCE in blood (A) and TCA in plasma (B) of 20-day-old rat pups. Two groups (n = 21 and 30) of pups were exposed to 600.0 ppm TCE vapor for 4 hr.

indicating a dose-rate dependence for the conversion of TCE to TCA. The plasma TCA elimination rate constant determined from the intravenous dosing studies was optimized after setting the value of P_0 to 0.27, and an apparent volume of distribution to 0.568 liter/kg. The apparent volume of distribution determined from iv administration was increased slightly from 0.541 to 0.568 liter/kg for the inhalation exposure. The estimated plasma elimination rate constant, based on the 600.4-ppm TCE exposure, was 0.063 $\pm$ 0.005 hr^{-1}.

Rat pups were not intravenously dosed with TCA; thus, the value of P_0 and kinetic constants for TCA were estimated from only the 600.0-ppm inhalation exposure. The apparent volume of distribution for TCA in the pup was set equal to that for the naive female Fischer 344 rat, 0.511 liter/kg (our unpublished data), and modeling attempts allowed either P_0 or the plasma elimination rate constant to vary. The proportion of TCE converted to TCA was estimated to be 0.12 by visual inspection. After setting P_0 to a value of 0.12, the plasma elimination rate constant was optimized to 0.014 $\pm$ 0.002 hr^{-1}.

Repeated TCE Inhalation Exposures

The single inhalation exposures with the lactating dams did not provide kinetic information on the transfer of TCA from the maternal blood supply to the milk compartment. Consequently, the repeated inhalation exposure with lactating dams served two important purposes. It was used (1) to test the fidelity of the repeated-exposure PB-PK model for predicting both TCE concentrations in the dam (Figs. 5A and B) and TCA concentrations in the dam (Figs. 6A and B) and pup (Fig. 6B), and (2) to estimate the bidirectional transfer coefficient for TCA from the maternal blood to the milk (Figs. 6A and B).

Under flow-limited conditions, the TCA concentrations in the milk were overestimated. Thus a diffusion-limited process was used to describe the TCA concentration time course in the milk. The TCA transfer coefficient [P_A, Eq. (7), Appendix II] was estimated by adjusting the value of P_A until an adequate correspondence was obtained between predicted and observed concentrations of TCA in the milk (Fig. 6B). The P_A value, based on the repeated inhalation exposure of 610 ppm TCE, was 0.0005 liters/hr. The PB-PK lactation model with the transfer coefficient allowed prediction of the time courses for TCE and TCA in the dam and pup for the drinking water exposure regimen. All TCE and TCA time courses for the repeated inhalation exposure represent predictions of the PB-PK

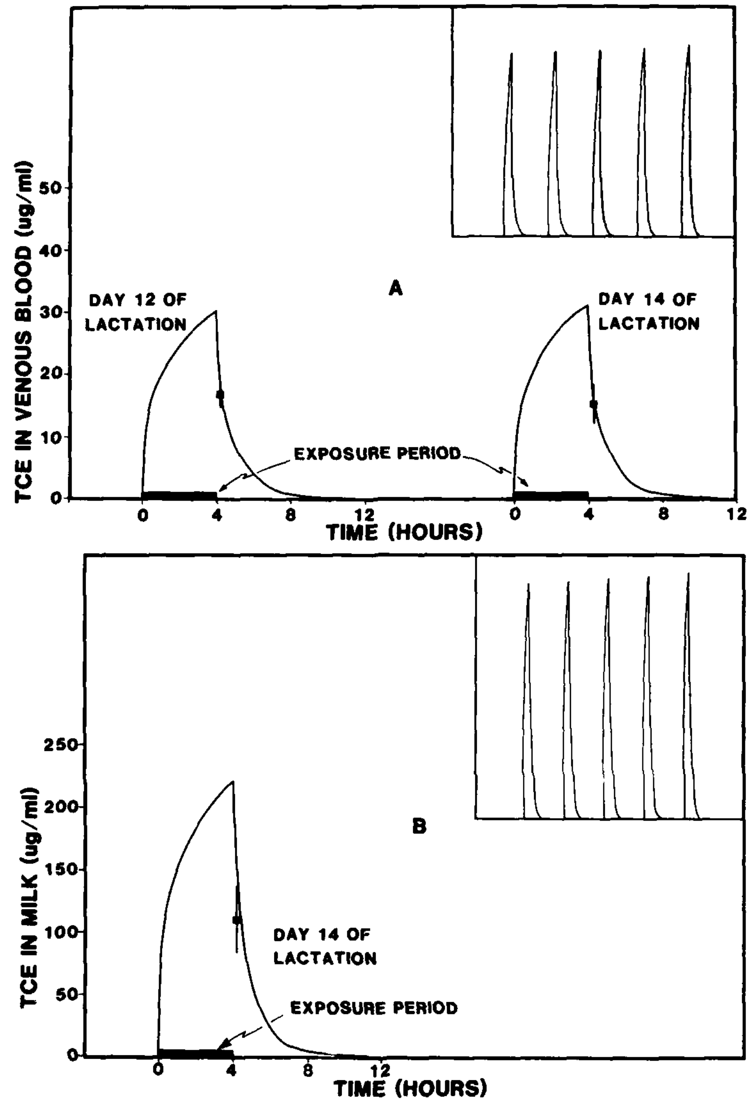

FIG. 5. Comparison of predicted (solid continuous lines) and experimental (squares) concentrations of TCE in venous blood (A) and milk (B) of lactating rats. Lactating rats were exposed to TCE vapor 4 hr/day, 5 days/week, for 2 weeks. In the upper right-hand corner is a portion of the 2-week simulation (Days 8–12 of lactation); the shaded area represents a tissue collection day. Three dams were milked and blood collected for TCA analysis 20 hr after exposure on Day 11 of lactation. Blood was collected immediately after exposure ($n = 3$) on Day 12 of lactation and blood and milk were collected immediately after exposure ($n = 5$) on Day 14 of lactation for TCE and TCA analyses.

lactation model except for the fitted maternal milk concentrations of TCA.

Trichloroethylene Exposure during Lactation

The experiments in this study were not designed to provide detailed time course information for TCE and TCA in the dam and pup during lactation but, rather, to develop a generalized PB-PK model structure for the lactating rat and nursing pup with limited *in vivo* experimentation. Once the PB-PK model structure was in place, the fidelity of the PB-PK model could be tested by compar-

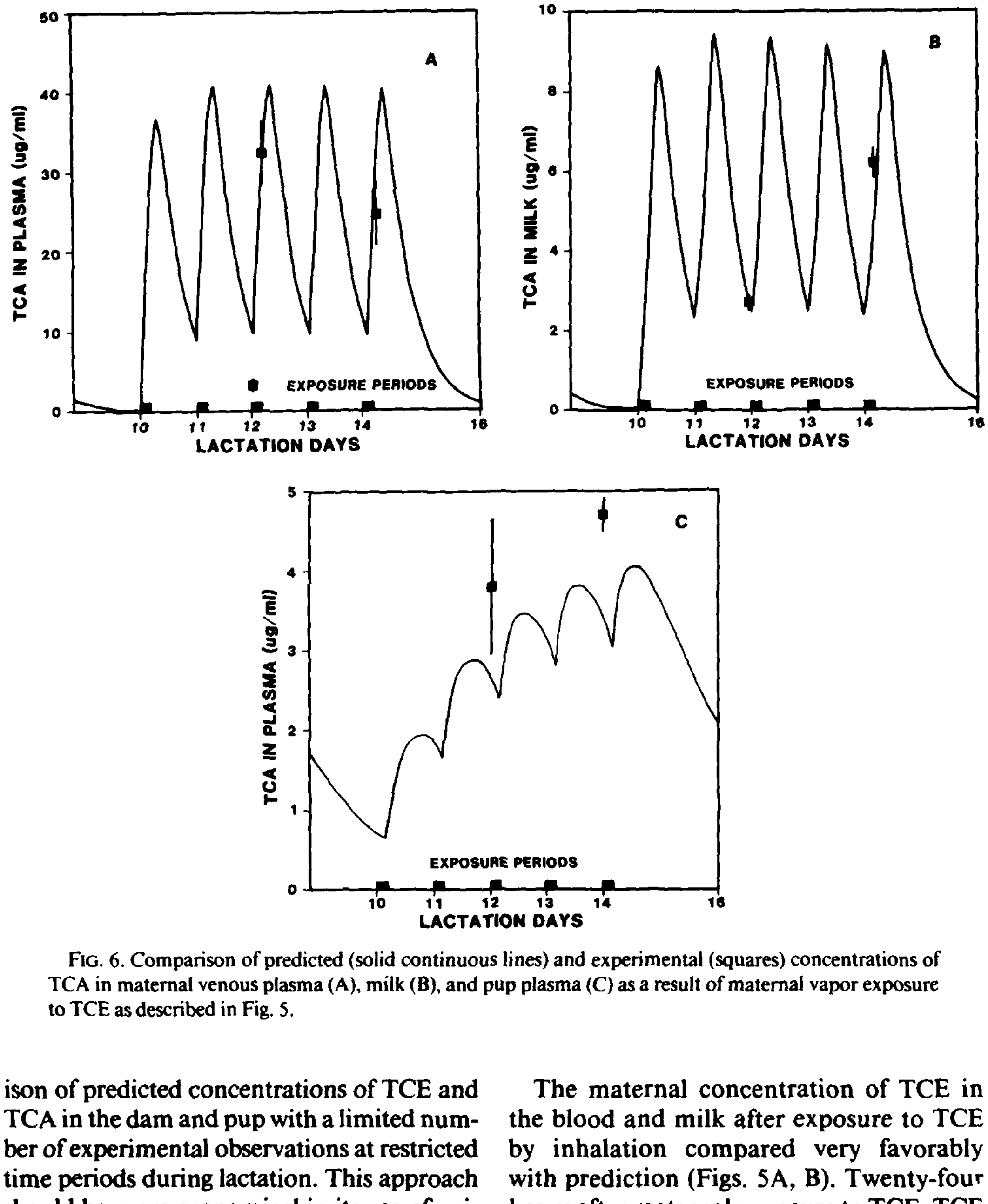

FIG. 6. Comparison of predicted (solid continuous lines) and experimental (squares) concentrations of TCA in maternal venous plasma (A), milk (B), and pup plasma (C) as a result of maternal vapor exposure to TCE as described in Fig. 5.

ison of predicted concentrations of TCE and TCA in the dam and pup with a limited number of experimental observations at restricted time periods during lactation. This approach should be more economical in its use of animals. Even though every attempt was made to limit the number of animals involved, significant numbers of pups and several dams were killed for analyses of TCE and TCA to ascertain the PB-PK model's ability to provide a satisfactory correspondence between predicted and observed concentrations of TCE and TCA in dam and pup.

The maternal concentration of TCE in the blood and milk after exposure to TCE by inhalation compared very favorably with prediction (Figs. 5A, B). Twenty-four hours after maternal exposure to TCE, TCE was not detected in pup blood. This observation was consistent with model prediction which was below the limits of detection for TCE (<0.03 μg TCE/ml blood). TCE was not detected in maternal blood or milk or pup blood for the drinking water-exposed animals. Again, model prediction for TCE concentrations in these tissues were also below the limits of detection for the assay procedure.

Trichloroacetic Acid Exposure during Lactation

The maternal TCA plasma concentrations, predicted on the basis of kinetic experiments with TCA, were in good agreement with observed values (Fig. 6A) immediately after exposure and within a factor of 2 at 20 hr after exposure. The simulated TCA concentrations in the milk of dams exposed to TCE by inhalation (Fig. 6B) are fitted results obtained by adjusting the transfer coefficient (P_A) and therefore do not represent model predictions. The model slightly underpredicted the TCA concentrations in pup plasma, but model predictions and observed values were considered to be in good agreement (Fig. 6C). For the drinking water study, model prediction was not quite as good. Maternal TCA plasma concentration predictions were consistently high, by a factor of about 2 (Fig. 7A). Predicted TCA concentrations in milk were also slightly above observed TCA concentrations in two of three samplings (Fig. 7B). Predicted pup plasma TCA concentrations were fairly consistent with observed values (Fig. 7C). The maternal yield of TCA from oxidation of TCE was set to 0.17 for the drinking water study because the dose rate of TCE was low and was similar to that for the 27-ppm inhalation exposure study. The predicted venous blood concentration of TCE in liver based on a 6-hr zero-order drinking rate was 0.15 and for the 27-ppm inhalation exposure 0.06 mg TCE/liter blood. This compares to 30 mg TCE/liter blood for the 600.4-ppm inhalation exposure. The computer-generated dotted lines in Figs. 7A, and B and C were generated assuming that only 10% (P_0 = 0.10) of the TCE is oxidized to the acid. This provided better fits for the maternal plasma concentrations of TCA but difficulties are still encountered with the milk and pup plasma concentrations of TCA.

For comparison purposes, peak measured concentrations of TCA in the maternal plasma and milk of the drinking water exposures were 2.3 μg TCA/ml plasma and 1.5 μg TCA/ml milk, and, in the inhalation exposures, 33.0 μg TCA/ml plasma and 6.1 μg TCA/ml milk. Peak measured concentrations of TCA in pup plasma were 1.5 μg TCA/ml plasma for the drinking water exposures and 4.8 μg TCA/ml plasma for the inhalation exposures.

DISCUSSION

Determining an infant's exposure to chemicals from ingestion of milk containing xenobiotics is of considerable toxicological interest for ensuring the well-being of the neonate. Breastfeeding of infants is again popular (Wilson *et al.*, 1980) and there is an increasing number of lactating women occupationally exposed to chemicals in the workforce. Wolff (1983) has reviewed the literature on occupational exposure of lactating women to metals, solvents, and halogenated hydrocarbons. Perhaps the most studied class of compounds in breast milk is drugs. Wilson *et al.* (1980) has reviewed the literature on these compounds. Unintentional environmental exposure of lactating women to chemicals appears substantial. Rogen *et al.* (1987) performed an epidemiologic study on 853 children exposed to PCBs from breast milk. Volatile organics are commonly found in the milk of lactating women living in urban settings (Pellizzari *et al.*, 1982). In West Germany, Niessen *et al.* (1984) found many children with residues of chlorinated hydrocarbons and PCBs. In Japan, Tojo *et al.* (1986) found chlordane in many milk samples from nonoccupationally exposed women.

Several interesting experimental findings resulted from our studies designed primarily for development of the PB-PK model. For example, metabolic capacities for oxidation of TCE are dependent on the dam's physiological state. The V_{max} value obtained for TCE in the lactating female rat (9.26 mg/kg/hr) is similar to that in the pregnant rat [9.18 mg/kg/hr (Fisher *et al.*, 1989)]. Both of these V_{max}

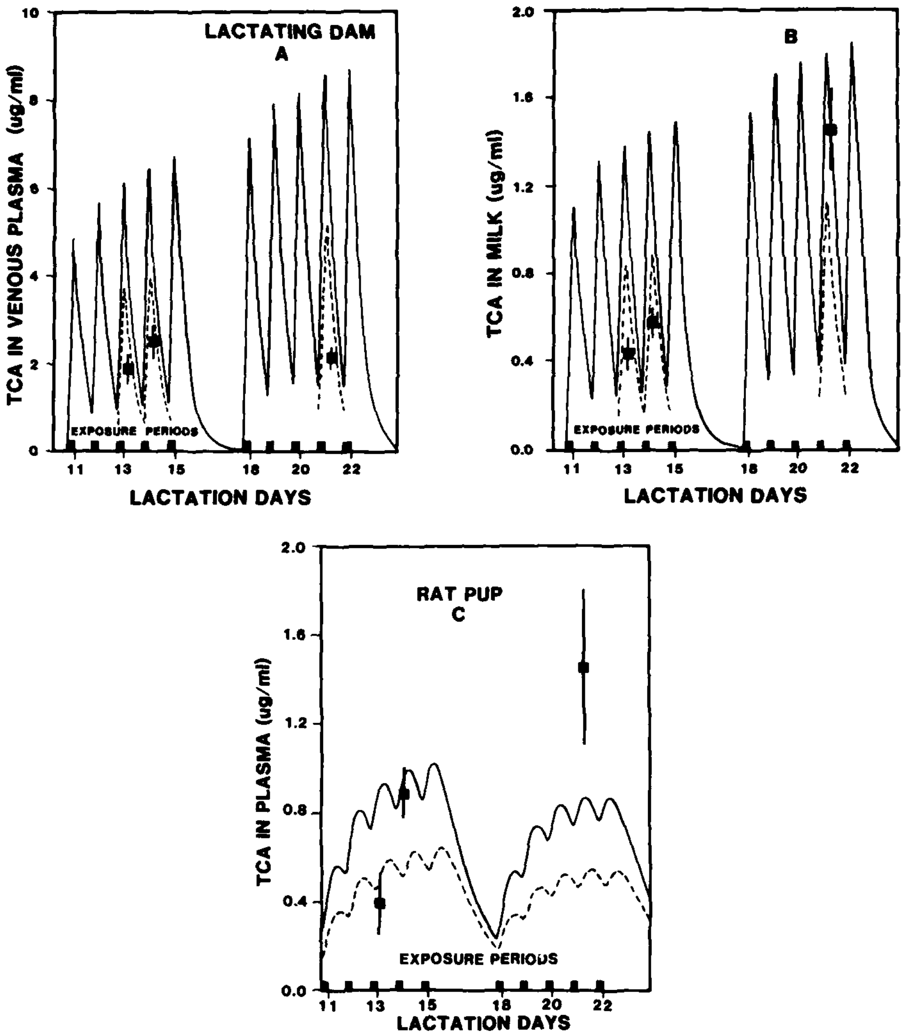

FIG. 7. Comparison of predicted (solid and dashed lines) and experimental (squares) concentrations of TCA in maternal venous plasma (A), milk (B), and pup plasma (C). PO, the proportion of TCE converted to TCA, equals 0.17 for the solid lines and 0.10 for the dashed lines. Lactating rats were provided daily drinking water containing an initial mean concentration of 333 μg TCE/ml water, 5 days/week, for 3 weeks. TCE-water consumption increased as lactation progressed. On Days 13 (n = 5), 14 (n = 5), and 21 (n = 4) of lactation, maternal blood and milk and pup blood were collected for TCA analysis.

values are less than the V_{max} value for TCE in the naive female [10.98 mg/kg/hr (Fisher *et al.*, 1989)]. With young male and female rat pups we found a relatively high V_{max} value for TCE (12.94 mg/kg/hr). The 21-day-old rat pup's ability to metabolize TCE is greater than that of the adult female and male [11.0 mg/kg/hr (Andersen *et al.*, 1987)] Fischer 344 rat. Another observation was that formation of TCA from TCE oxidation is dependent on the dose rate in the lactating rat. Proportionally less TCA is produced at low exposure concentrations of TCE than at high exposure concentrations. Conversely, DeKant *et al.* (1986) reported that 17% of a 2 mg/kg single oral dose of TCE was converted to TCA, and a slightly lower yield of TCA (14%) was obtained with 20 and 200 mg/kg doses in female Wistar rats. Clearance of TCA in female rats was also dependent on their physiological state. The plasma half-life for TCA is 6.8 hr in a 0.200-kg lactating rat (not including loss due to pup suckling) and 9.5 hr for a 0.200-kg pregnant rat (Fisher *et al.,* 1989). Consistent with observations on rat pups dosed with sulfobromophthalein (Klaassen, 1973) we found that the rat pup's (0.025 kg) plasma half-life for TCA (16.5 hr) is substantially greater than that of the mature rat.

The transfer of TCE to the milk compartment from systemic circulation in the dam was modeled as a flow-limited process [Eq. (2), Appendix II] with the blood being in intimate contact with the milk gland (Fig. 1). Thus, TCE enters the milk compartment at a rate of $Q_{mt} * C_a$ and leaves at a rate of $Q_{mt} * (C_{milk}/P_{milk})$. TCA distribution, however, was modeled as a diffusion-limited process [Eq. (7), Appendix II] in which blood flow carried TCA to the mammary tissue and then TCA diffused across the mammary tissue in the milk compartment (Fig. 1). With this description, transfer of TCA into the milk is dependent on the rate at which TCA diffuses across the mammary tissue and not blood flow to the mammary tissue alone. Thus, TCA enters the milk compartment at the rate $P_A * (C_{mt}/P_{mt})$, and back-diffuses at the rate $P_A * C_{mk}$. The clearance term for TCA, P_A (Fig. 1), is 0.0005 liter/hr, based on fitting the milk concentrations of TCA (Fig. 6B) from the repeated inhalation exposure.

We described the pup's suckling of the milk simply as a zero-order process in which the pup litter suckling rate was equal to the milk production rate (K_{milk}). Thus, theoretically, the size of the milk compartment remains constant and was set to 0.002 liter. This approach may not be appropriate for human lactation models because of differences in nursing schedules. The rates of ingestion of TCE and TCA by the pup litter are described as $K_{milk} * C_{milk}$ and $K_{milk} * C_{mk}$, respectively. Model predictions compared quite favorably with experimental observations for the inhalation exposure and somewhat less favorably for the drinking water exposure. Drinking water studies are more difficult because the exposure is not controlled. Attempts to model a repeated gavage dosing study during lactation were not successful. TCE concentrations in the maternal blood were below model predictions as were TCA concentrations in the maternal milk and pup plasma. This was surprising since this was not the case with the pregnant rat (Fisher *et al.,* 1989). Recent modeling attempts of gavage dosing studies with TCE suggest that absorption of TCE across the gastrointestinal tract is multiphasic (Fisher *et al.,* 1990; Staats and Conolly, 1989). Perhaps the complexity of gut absorption of TCE and the dose-rate-dependent formation of TCA from oxidation of TCE in the lactating rat contributed to the difficulty of modeling oral intubation.

An important use of the PB-PK lactation model is to compare the TCE and TCA exposure profiles of the dam with the exposure profiles of the pup. The predicted TCE concentrations in the pup were not examined experimentally, and therefore are considered theoretical. On the other hand, the predicted TCA concentrations in the pup were examined experimentally. Table 3 shows the exposure estimates for TCE and TCA in the dam and pup for both routes of exposure. The model predicted cumulative area under the curve (AUC) estimates for TCE in blood and for TCA in plasma served as a means of comparing maternal and pup exposures to these chemicals. Individual pups were exposed to only a small fraction of the dam's exposure to TCE. Pup blood AUC values for TCE were less than 2% of the maternal venous blood AUC values for the inhalation- and drinking water-exposed animals for 3 weeks of lactation. Pup plasma AUC values for TCA were 30 and 15% of maternal AUC values for the drinking water- and inhalation-exposed animals, respectively, for 3 weeks of lactation (Table 3). Thus, the pup is exposed primarily to the oxidative metabolite TCA as a result of maternal exposure to TCE. Only 6.8 and 4.2% of the TCA produced from oxidation of TCE in the drinking water- and inhalation-

TABLE 3

MODEL-DERIVED EXPOSURE ESTIMATES FOR TCE AND TCA DURING 3 WEEKS OF LACTATION

	Route of exposure	
	Drinking water	Inhalation
Dam		
TCE metabolized (mg)	66.7	305.5
TCE exhaled (mg)	2.6	752.5
TCE in maternal blood, AUC (mg/hr/liter)	6.5	1,735.3
TCE in milk, AUC	42.2	12,623.7
TCA in plasma, AUC	1946.6	8,798.5
TCA in milk, AUC	487.6	2,175.6
Pup[a]		
TCE ingested by each pup (mg)	0.010	1.500
TCE metabolized by each pup (mg)	0.006	0.465
TCE exhaled by each pup (mg)	0.004	1.035
TCE in pup venous blood, AUC	0.092	29.092
TCA ingested to each pup (mg)	0.110	0.499
TCA in pup plasma, AUC	584.8	1,316.14

[a] These exposure estimates are based on a litter size equal to 7.

exposed dams, respectively, were eliminated by lactational transfer. The calculated contribution of TCA exposure from metabolism of TCE in the pup is small, 0.7% for the drinking water-exposed rats and 11% for the inhalation-exposed rats. For the drinking water and inhalation exposures the predominant tissue exposure for the dam and the pup was TCA (Table 3). The administered dose of TCE for the inhalation exposure was about 15 times greater than the drinking water exposure based on the simulated amounts of TCE exhaled and metabolized (Table 3) by the lactating rats. During maternal exposure to TCE the fetus is exposed to both the parent compound (TCE) and its oxidative metabolite (TCA), *in utero* (Fisher *et al.*, 1989), and after birth, the exposure to pups is primarily from the oxidative metabolite. These findings suggest that TCE exposure during pregnancy is of greater toxicologic concern than during lactation.

This study is an initial attempt at developing a generic PB-PK lactation model to describe the lactational transport of a volatile, lipophilic chemical and its persistent hydrophilic metabolite. This PB-PK lactation model, with its limited experimental observations, did predict TCA concentrations in pups within about a factor of 2, which is considered very good. The PB-PK lactation model developed in this study has, perhaps, more important applications with chemicals that are nonvolatile, lipophilic, and persistent in the environment, such as polychlorinated biphenyls.

APPENDIX I: LACTATION MODEL NOMENCLATURE

TCE and TCA

C_a	Arterial blood concentration (mg/liter)
C_{v_i}	Venous blood concentration leaving ith tissue (mg/liter)
C_i	Concentration in ith tissue (mg/liter)
P_i	Tissue i/blood partition coefficient (liters blood/liters tissue)
Q_i	Blood flow to ith tissue (liters/hr)
V_i	Volume of ith tissue (liters)
V_d	Volume of distribution (liters)
K	Elimination rate constant (per hr)
P_0	Proportionality constant (unitless)
P_A	TCA transfer coefficient (liters/hr)
SC	Stoichiometric conversion factor (unitless)
A_i	Amount in ith tissue (mg)
K_{milk}	Zero-order pup litter suckling rate (liters/hr) equals milk production rate

Subscripts i

l	liver
mt	mammary tissue

milk milk for TCE
pla maternal plasma
mk milk for TCA

APPENDIX II

TCE Equations

The tissue mass balance equations for TCE in the lactating dam and pup are formulated as outlined in Ramsey and Andersen (1984). The rate of change in the amount of TCE in the milk compartment of the lactating dam comprises two terms, one related to the maternal blood circulation and partitioning to the milk compartment, and the other, to pup suckling:

$$dA_{\mathrm{milk}}/dt = Q_{\mathrm{mt}}(C_{\mathrm{a}} - C_{\mathrm{milk}}/P_{\mathrm{milk}}) - dA_{\mathrm{suck}}/dt. \quad (2)$$

The mammary tissue is omitted from this description; thus the maternal blood supply is considered to be in intimate contact with the milk. The second term is identical to the pup litter suckling rate and is used for estimating each pup's exposure to TCE,

$$dA_{\mathrm{suck}}/dt = K_{\mathrm{milk}} * C_{\mathrm{milk}}, \quad (3)$$

where K_{milk} is the zero-order pup litter suckling rate. The concentration of TCE in the milk (C_{milk}) is the integration of Eq. (2) divided by the volume of milk (0.002 liter).

TCA Equations

The kinetics of TCA in the dam are described by a hybrid classical one-compartment model (Fisher *et al.,* 1989). The rates of TCA production in the lactating rat and developing pup are expressed as a proportion (P_0) of the rate of TCE metabolism. The P_0 value is either 0.27 or 0.17 for the lactating rat, depending on the TCE exposure (see Results), and 0.12 for the rat pup. A stoichiometric conversion factor (SC) was used to adjust the molecular weight for TCA. This increase in molecular weight is the result of enzymatic conversion of TCE to the oxidized TCA metabolite:

$$dA_{\mathrm{tca}}/dt = P_0 * \frac{V_{\max} * C_{\mathrm{vl}}}{K_m + C_{\mathrm{vl}}} * \mathrm{SC}. \quad (4)$$

The rate of change in the amount of TCA in the maternal plasma is described by the production term for TCA [Eq. (4)], a first-order plasma elimination term, and a term describing the blood flow and partitioning to the mammary tissue:

$$dA_{\mathrm{pla}}/dt = dA_{\mathrm{tca}}/dt - V_{\mathrm{d}} * K * C_{\mathrm{tca}} - Q_{\mathrm{mt}}(C_{\mathrm{tca}} - C_{\mathrm{mt}}/P_{\mathrm{mt}}). \quad (5)$$

The concentration of TCA in the maternal plasma is

$$C_{\mathrm{tca}} = A_{\mathrm{tca}}/V_{\mathrm{d}}, \quad (6)$$

and A_{tca} is the integral of Eq. (5).

In the mammary tissue, TCA was considered to be flow limited with respect to maternal blood flow perfusing this tissue. Movement of the TCA across the mammary tissue from the blood supply into the milk compartment was modeled as a diffusion-limited process. The concentration of TCA available for diffusion across the mammary tissue is the calculated venous blood concentration in the mammary tissue. P_{A}, the transfer coefficient for TCA, is initially set to 0.0005 liter/hr and increases in value proportionally with the growth of the mammary tissue during 22 days of lactation (9.6%). The equation for the mammary tissue is

$$dA_{\mathrm{mt}}/dt = Q_{\mathrm{mt}}(C_{\mathrm{tca}} - C_{\mathrm{mt}}/P_{\mathrm{mt}}) - P_{\mathrm{A}}(C_{\mathrm{mt}}/P_{\mathrm{mt}} - C_{\mathrm{mk}}). \quad (7)$$

The milk compartment is described with two terms. One term is related to the diffusion of TCA across the mammary tissue into the milk and the other term is related to loss of TCA from the milk by suckling:

$$dA_{\mathrm{mk}}/dt = P_{\mathrm{A}}(C_{\mathrm{mt}}/P_{\mathrm{mt}} - C_{\mathrm{mk}}) - dA_{\mathrm{suck}}/dt. \quad (8)$$

As with TCE, the second term is identical to the pup litter suckling rate and is used for estimating each pup's exposure to TCA:

$$dA_{suck}/dt = C_{mk} * K_{milk}, \quad (9)$$

where C_{mk} is the concentration of TCA in the milk. C_{mk} is the integration of Eq. (8) divided by the volume of milk (0.002 liter).

The rate of change in the amount of TCA in the pup plasma is described in a fashion similar to that for the dam [Eq. (5)] except the mammary tissue flow term is not included. Equation (9) is used to describe the ingestion of TCA.

Equations for the inhalation exposure are reported in Ramsey and Andersen (1984) and for the water drinking exposure in Fisher *et al.* (1989). For modeling the repeated inhalation exposures, the suckling rate constant, K_{milk}, is set to zero during the inhalation exposures because the pups are separated from the dams. Also, during the inhalation exposure the maternal milk compartment is assumed to increase in a linear fashion:

$$V_{milk} = 0.002 + K_{milk}(t), \quad (10)$$

where $0 \leq t$ (time) ≥ 4 hr. At the end of exposure, the rate at which pups suckle milk is assumed to increase exponentially ($t\frac{1}{2} = 1$ hr) and the volume of milk decreases exponentially ($t\frac{1}{2} = 1$ hr) until the suckling rate constant and volume of milk return to their preexposure values.

The lactating dams used for the repeated-exposure studies were previously exposed to TCE during pregnancy (Fisher *et al.*, 1989). Residual amounts of TCA in dams and pups were estimated from the pregnancy and lactation models. On Day 3 of lactation, the beginning of the lactation exposure studies, the dam's body burden of TCA was estimated to be 0.35 mg from inhalation exposure to TCE and 0.10 mg from drinking water exposure to TCE during pregnancy. Each pup's body burden of TCA was 0.0069 mg TCA for the inhalation exposure and 0.0022 mg TCA for the drinking water exposure.

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The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Uses of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, DHHA, National Institute of Health Publication 85-23, 1985, and the Animal Welfare Act of 1966, as amended.

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